Pharmacogenetics

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Introduction

The individual response to drug therapy may vary greatly, making the success of a given treatment strategy difficult to predict, particularly because multiple factors influence drug response. In addition to age sex, comorbidity, renal and hepatic function, the role of genetic factors that determine an individual's response to drug treatment is increasingly being realized. Genetic differences influencing the pharmacokinetics of a drug may exist at the level of drug metabolism determined by several well characterised phase I and phase II enzymes, with cytochrome P450 being the most important metabolizing system. Drug disposition is not only influenced by metabolizing enzymes but also by transport proteins, that play an important role in the processes of drug absorption, distribution and excretion. In addition to pharmacokinetic determinants pharmacodynamic effects of a drug may be determined by genetic differences in the drug target. Although to date an increasing number of genetic polymorphisms (SNPs) in single genes has been associated with variability of protein function and drug disposition, dose-response is generally influenced by a whole spectrum of genes, making analysis of a single gene insufficient to explain the variability of a pharmacological effect. Despite this complex interaction of factors determining drug response, several examples of direct genotype-phenotype relations have been discovered, that have led to the development of routine diagnostic tools for the individualization of drug dosing regimens.

Drug metabolizing enzymes

Drug metabolizing enzymes are subdivided into two categories: phase-I and phase-II enzymes. Phase I enzymes are responsible for the oxidative degradation (oxidation, reduction, hydrolysis) of endogenous substances (e.g. steroids) and xenobiotics. Phase-II enzymes render the coumpounds more water-soluble mainly by glucuronidation, sulfation, acetylation and methylation. The most important phase I enzyme system is cytochrome P450 (CYP), that metabolizes the majority of pharmacological compounds (50-60%). As many as 57 CYP genes

have been characterized, among these only the enzymes encoded by CYP1, CYP2 and CYP3 genes seem to contribute to the metabolism of drugs [1]. Almost all of the CYP isoforms possess genetic polymorphisms, the CYP2 family exhibiting the highest genetic variability with a distinct clinical impact. CYP2D6 is the best characterized isoenzyme and is responsible for the metabolism of most antipsychotics and antidepressants and several antiarrhythmics. In 6-10% of Caucasians and 1% of Asians the enzymatic activity is completely absent resulting in a "poor metabolizer" (PM) phenotype and leading to increased exposure and toxicity of drugs metabolized by CYP2D6 [2]. Some individuals possess multiple copies of the gene expressing the ultra-rapid metabolizer (UM) phenotype resulting in subtherapeutic plasma concentrations at normal doses [3]. About 3% of Caucasians carry the CYP2C9 or CYP2C19 PM phenotype predisposing them to an increased anticoagulatory effect of s-warfarin and increased toxicity of the antiepileptic drug phenytoin. For many drugs conjugation by phase-II enzymes represents the main metabolic pathway, although these are not as well characterized as the oxidative enzymes. Nevertheless a few important polymorphisms have been identified. Thiopurine S-methyltransferase (TPMT) catalyzes the S-methylation of azathioprine (AZA), 6-mercapto-purine (6-MP) and thioguanine. TPMT activity exhibits genetic polymorphisms in 10% of Caucasians, with 1/300 individuals having a complete deficiency that leads to an increased risk for fatal myelosuppression [4]. Toxicity of the antituberculostatic agent isoniazid has been associated with a reduced activity of the enzyme N-acetyltransferase-2. Life-threatening diarrhea and leukopenia is observed in up to 25% of patients receiving the anti cancer drug irinotecan and has been related to polymorphisms in the uridine diphosphate-glucuronosyltransferase (UGT)-1A1 gene [5]. For most of the described enzymes routine genotyping is established which makes an individual dose adaptation possible.

Drug transporters

An increasing list of transport proteins have been found to play an important role in regulating drug uptake from intestine and sinusoidal blood into the liver, in regulating and limiting drug distribution to different body compartments like central nervous system and in controlling drug efflux into bile and renal tubule [6]. Thus genetic polymorphisms in human membrane transporters may also contribute to interindividual differences in drug response. The transport proteins described to date that are involved in transmembrane drug transport include the ATP-binding cassette (ABC) transporters MDR1 (multidrug resistance protein 1), BSEP (bile salt export pump), MRP1, 2, 3 and 4 (multidrug resistance associated protein 1, 2, 3 and 4) and BCRP/ABCG2 (breast cancer resistance protein) found in the basolateral or canalicular membrane of liver cells and some of them in the kidney. Organic anion and cation transporters

like the organic anion transporting polypeptides (OATP) are mainly responsible for drug uptake into the hepatocyte and the organic anion and cation tranporters (OAT/OCT) for the renal tubular secretion and reabsorption of drugs. Multiple SNPs and haplotypes have been characterized in the MDR1 gene (*ABCB1*) and its gene product p-glycoprotein (p-gp), which has been recognized to play a primary role in drug disposition [7]. Although data on the functional effect of the variant MDR1-alleles are not always conclusive and sometimes contradictory, the SNP 3435TT in exon 26 was associated with reduced intestinal expression of p-gp. Oral bioavailability of digoxin, the most extensively studied p-gp substrate, was significantly higher in individuals carrying the genotype 3435CC [8]. Enhanced p-gp activity was found in subjects with the 3435TT/G2677T genotype, causing a 40% reduction in AUC values following oral administration of the p-gp substrate fexofenadine, when compared to the 3435CC genotype [9]. MRP2 plays a central role in the biliary excretion of organic anions such as many anticancer drugs. Recently a mutation in exon 10 of the MRP2 gene was shown to confer loss of transport activity, leading to methotrexate overdose with renal failure [10]. The organic anion transporting polypeptide 1B1 (OATP1B1) and multidrug resistance-associated protein 2 (MRP2 / *ABCC2*), are thought to be the major transporters determining in the pharmacokinetics of pravastatin in humans. A genetic polymorphism in the OATP1B1 (*SLCO1B1*) gene led to a reduced total and renal clearance of pravastatin in healthy Japanese individuals [11].

It is now evident that hepatocellular transport proteins are also important in determining the exposure of the liver to drugs and they may therefore be involved in the onset of drug induced liver injury[12, 13]. Reduced canalicular efflux transport protein activity can lead to intracellular bile acid and drug accumulation resulting in liver cell damage. A polymorphism in BSEP/ABCB11 with a valine-to-alanine exchange in the highly conserved amino acid position 444 of the BSEP protein has been associated with a decreased hepatic content of BSEP [14]and with a threefold increased risk to develop cholestatic drug side effects [15].

Drug targets

Genetic polymorphisms in drug targets can have a major influence on the effect of a drug. To date more than 20 examples of drug targets with genetic variability that directly influences drug response have been identified. The anticoagulatory effect of warfarin is determined on the one hand by the genotype and activity of the metabolizing enzyme CYP2C9 and on the other hand by the genetic variability of the target enzyme Vitamin K epoxide reductase (VKOR) [16]. Homozygous mutations in the β1-adrenergic receptor reduces sensitivity to beta blocking agents in the treatment of arterial hypertension [17] and a correlation has also been seen between mutations in the β2-adrenergic receptor and response to oral β2-agonists [18]. Interindividual

response and increased toxicity of psychotropic substances such as antidepressants and antipsychotics can be defined by the genotype of the corresponding receptor type [19, 20]. Recently several examples of genetic variability in proteins targeted by monoclonal antibodies have been found to cause an insufficient drug effect. A study with 200 patients suffering from Crohn's Disease showed a significant association between the biological response to infliximab, a chimeric antibody binding tumor-necrosis-factor-α, and a single nucleotide polymorphism in the FCGR3A gene, which codes for a receptor for the Fc fragment of IgG (FccR) on natural killer cells and macrophages [21]. In breast cancer therapy, copy number or expression levels of the HER2 gene that encodes a transmembrane tyrosine kinase receptor expressed on breast cancer cells, is highly associated with the therapeutic efficacy of the monoclonal antibody trastuzumab which inhibits the proliferation of tumor cells overexpressing HER2 [22]

Conclusion

The discovery of further genotype-phenotype associations will define the role of pharmacogenetics in future clinical practice. Genotype-based prescribing could improve response rate and decrease the socio-economic burden of adverse events. Nevertheless the effects of most drugs are determined by several proteins, and composite genetic polymorphisms in multiple genes coupled with non genetic factors often have a high impact on individual drug response. New strategies are therefore needed to identify, for a given drug, the relevant genes: these include (i) genome-wide haplotype mapping, (ii) gene expression analyses, (iii) proteomic methods, and (iv) candidate gene approaches based on known pharmacokinetic and pharmacodynamic factors. These approaches, coupled with evolving biostatistical models, will elucidate polygenic determinants of drug response. Clinical validation of these polygenic models requires large clinical trials of uniformly treated and systematically characterized patients, high-throughput genomic methods, and sophisticated bioinformatic analyses. These studies could yield an exciting new panel of molecular diagnostics (i.e. genotypes) that can be employed to improve drug therapy by reducing toxicity and increasing efficacy.

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